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The decline in battlefield mortality driven by advances in body armor technology has resulted in a concomitant increase in injuries to extremities requiring the use of prosthetics. The main limitation in deployment of prosthetic technology is the integration of the prosthetic device into the body.
The proposed solution to this problem involves merging tissue engineering and medical imaging technology to directly implant a prosthetic interface that will rapidly and securely integrate with surrounding bone and soft tissue. Through controlled placement of appropriate cells, signaling factors, and scaffold materials, this process will enable the generation of multi-component implants that include a prosthetic interface. The grand vision for such technology is the widespread deployment of tissue implants that use CT or MRI scans and robot-assisted surgery to guide the direct in vivo generation of

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FINAL REPORT
ENGINEERING BIOLOGICAL INTERFACES TO ENHANCE PROSTHETIC INTEGRATION

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Problem Statement
The decline in battlefield mortality driven by advances in body armor technology has resulted in a concomitant increase in injuries to extremities requiring the use of prosthetics. A main limitation in deployment of prosthetic technology is the integration of the prosthetic device into the body. Using current procedures, effective prosthetic integration often requires 18 months and multiple surgeries.

Long Range Goal
The proposed solution to this problem involves merging tissue engineering and medical imaging technology to directly implant a prosthetic interface that will rapidly and securely integrate with surrounding bone and soft tissue. Through controlled placement of appropriate cells, signaling factors, and scaffold materials, this process will enable the generation of multi-component implants that include a prosthetic interface. The grand vision for such technology is the widespread deployment of tissue implants that use CT or MRI scans and robot-assisted surgery to guide the direct in vivo generation of composite implants that provide a secure interface for any prosthetic device desired. This will provide a more functional prosthetic interface in a shorter time and enable the more rapid development and deployment of advanced prosthetic devices.

Proposed Tasks and Progress
Task 1: Deliver cell/material combinations that develop into tissue/implant composites that release bioactive molecules (8/06-1/07)

a. Demonstrate viability and sterility of implants; characterize biosynthetic behavior of cell/material composites
b. Evaluate multiple formulations of materials and cells to maximize tissue development and mechanical rigidity
c. Characterize patterns of release of bioactive factors from implants

Tasks 1a, 1b, and 1c were completed as planned. The base polymer system used for cell delivery was alginate, a natural biopolymer shown be successful in compatible for encapsulation of over 30 cell types. The cell type chosen for delivery in these applications was chondrocytes isolated from bovine articular cartilage. The bioactive molecule chosen for controlled release studies was insulin-like growth factor-I (IGF-I), a protein shown to be highly anabolic for many cell types including chondrocytes. To achieve controlled release of IGF-I, the alginate biopolymer was modified by grafting a peptide sequence capable of binding IGF-I. The peptide sequence KPLHALL derived from the hydrophobic pocket of the binding region of IGFBP-5 was covalently attached to alginate using aqueous carbodiimide chemistry. The modified alginate (KPL-alginate) was compared to unmodified alginate for its ability to bind IGF-I using surface plasmon resonance (SPR). Further, the effect of the KPLHALL sequence on release of IGF-I was determined by controlled release studies quantified by ELISA. Lastly, the effect of this modification on cell behavior was determined by evaluating proteoglycan synthesis by chondrocytes in the presence and absence of IGF-I in modified and control alginate gels.

SPR data (Figure 1) demonstrated that grafting of the KPLHALL sequence to alginate enhanced IGF-I binding to alginate by a factor of ~40, as indicated by a decrease in the dissociation constant k_D from 1 μM to 25 nM. As a result of this increase in binding, the effective diffusivity of IGF-I in modified gels decreased by a factor of 8 as compared control gels, which increased the characteristic time for release from 1.2 to 9 weeks (Fig 2). Models for release of IGF-I from alginate gels based on diffusion-reaction scenario described the data well and yielded binding constants for control and modified alginate that were consistent with SPR data. Data from function studies of chondrocyte metabolism demonstrated that the modified polymer enhanced proteoglycan synthesis both in the presence and absence of IGF-I as well as extending the duration of action of the growth factor.

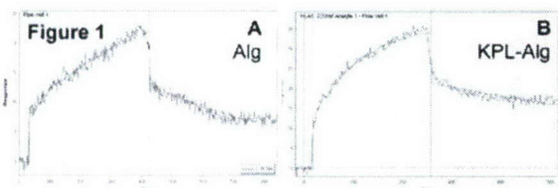


Table 1

	k_{on} (10^6/Ms)	k_{off} (10^{-3}/s)	k_D (nM)
IGFBP-5	1.2	4.4	3.7
Alg	0.003-0.009	1.1-2.2	220-1800
KPLHALL-Alg	0.03-0.12	0.07-1.5	25-57

Figure 1: Surface plasmon resonance (SPR) data for binding of IGF-I to unmodified alginate (Alg, left) and modified alginate (KPL-Alg, right). Kinetic constants (k_{on}, k_{off}) and equilibrium binding constants (k_D) for Alg and KPL-Alg are compared to the native IGFBP-5 protein from which the binding sequence was derived.

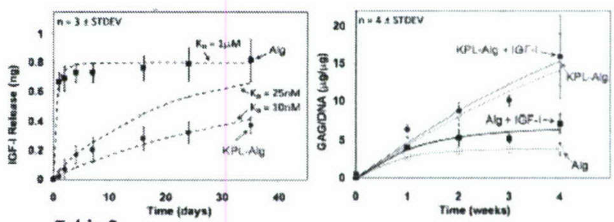


Table 2

	τ_{GF} (weeks)	D_{eff} (cm²/s)	τ_{GAG} (weeks)
Alg	1.2	2.8 x 10^{-7}	0.8 - 1.2
KPLHALL-Alg	9.4	3.5 x 10^{-8}	6.9 - 7.0

Figure 2: Controlled release data (left) and biosynthesis data (right) for Alg and KPL-Alg gels. In controlled release studies, the amount of IGF-I released from gels with time was compared to a diffusion-reaction model for a range of kD (dotted lines). The time constant for release (τ_{GF}) was used to calculate an effective diffusivity (D_{eff}) and compared to the time constant for glycosaminoglycan biosynthesis (τ_{GAG})

Task 2: Deliver implants that integrate with surrounding hard and soft tissues (1/07-11/07)

a. Place cell/material composites directly into defects in bone-biomaterial interface
b. Characterize development of new tissue implanted into defects into bone and soft tissue; examine integration between newly developed and existing tissue
c. Evaluate mechanical properties of implant-tissue interface

The primary focus of this effort was to develop an in vitro system for evaluating cells and materials for integrating prosthetic materials with tissues in the body, particularly bone. For the purpose of the current study, this involved engineering the interface between cancellous bone harvested from the bovine femur and implant-grade porous stainless steel, a good candidate material for a universal prosthetic interface. The material/cell delivery vehicle chosen for this application was collagen, which has a long history as a scaffold to support cell growth. Further, recent studies have developed methods for photocrosslinking of collagen using riboflavin and exposure to visible (458 nm) light. As such, this study focused on riboflavin-mediated photocrosslinking of and the ability to integrate stainless steel and cancellous bone.

The integration protocol utilized a Plexiglas mold to align cylindrical pieces of cancellous bone and the porous stainless steel (Figure 3). A collagen/riboflavin mixture was injected between the bone and stainless steel, with excess volume to ensure that the mixture penetrated the pores of both the bone and metal. The mold was then exposed to a 458 nm light source for times up to 160 seconds and samples were removed from the mold.

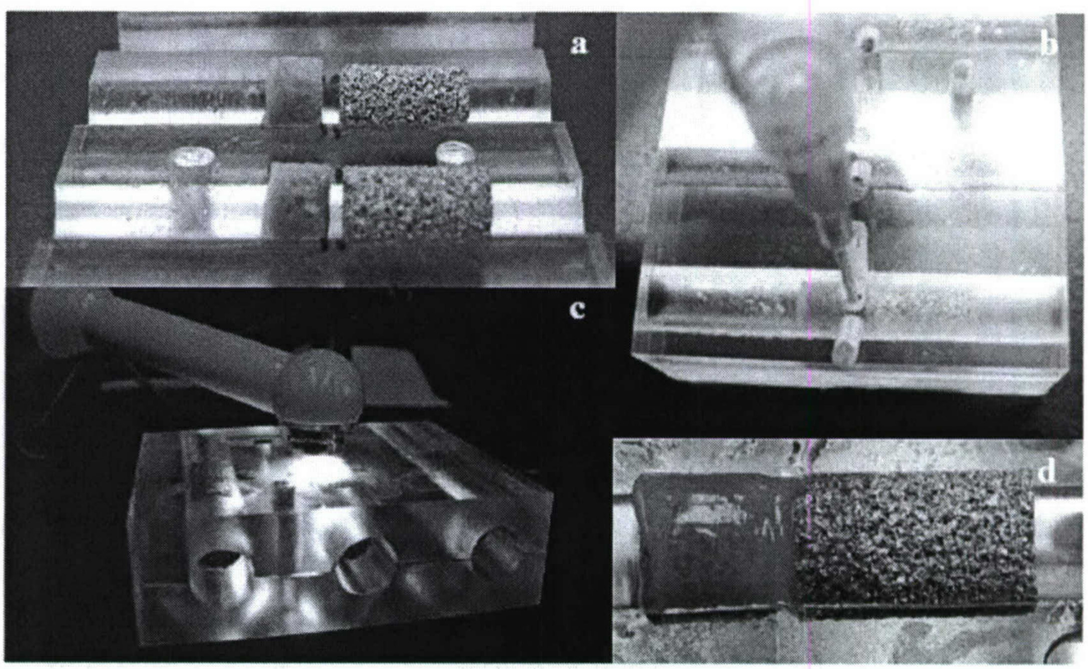
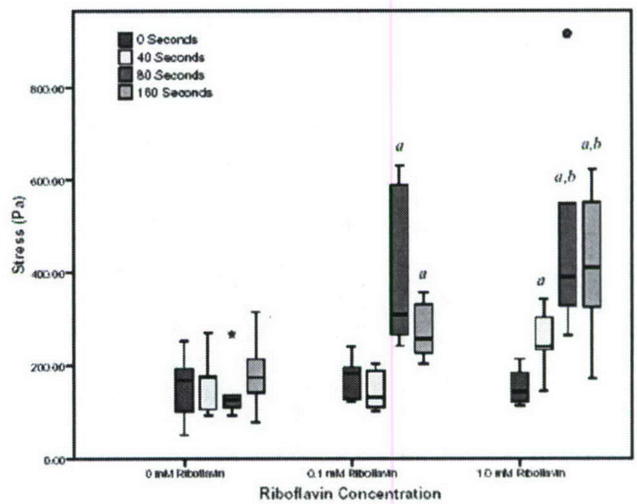


Figure 3: Generation of integrated bone-metal constructs. Cylindrical pieces of cut bone and machined stainless steel were separated by ~2mm placed in a Plexiglas mold (a). A collagen/riboflavin mixture was injected in the gap (b) and the mold was exposed to 458 nm light (c) prior to the removal of the samples.

To determine the extent to which riboflavin-mediated crosslinking enhanced the mechanical properties of collagen gels, the compressive modulus of samples was determined using an Enduratec ELF 2100 test frame for a range of riboflavin concentrations up to 1 mM and for a range of light exposure up to 160 seconds. The increase in compressive modulus was dependent on both time of exposure and riboflavin concentration (Figure 4). Maximal effect was seen at 1 mM riboflavin after 80 seconds of exposure, with a 2-4 fold increase in modulus.

Figure 4: Compressive modulus of photocrosslinked collagen gels using ranges of riboflavin up to 1 mM and light exposure times up to 160 seconds. Data are mean of n=8 samples with 90% confidence intervals (boxes) and standard deviations (error bars)



To determine enhancement of bone-metal integration, composite samples constructed as described above (Figure 3) were mounted in Enduratec ELF 2100 test frame and pulled to failure in tension (Figure 5). From the force-displacement curves, tensile modulus, ultimate tensile strength, strain energy density, and strain at failure were determined. Photocrosslinking did not enhance tensile modulus, but increased tensile strength by ~20%, failure strain by ~40%, and strain energy density by ~65% (Figures 6 and 7).

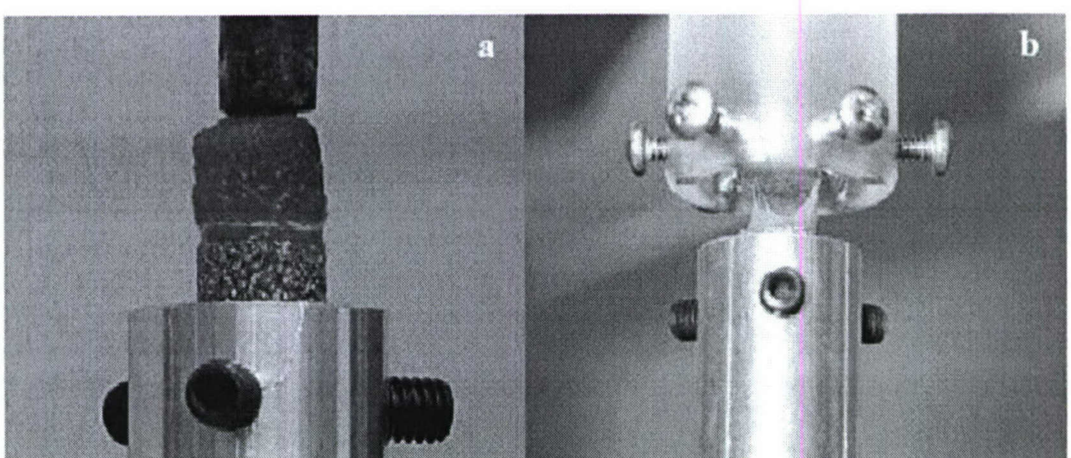


Figure 5: Tensile failure testing of bone-collagen-stainless steel composites. Samples were pulled to failure at a rate of 0.1 mm/s.

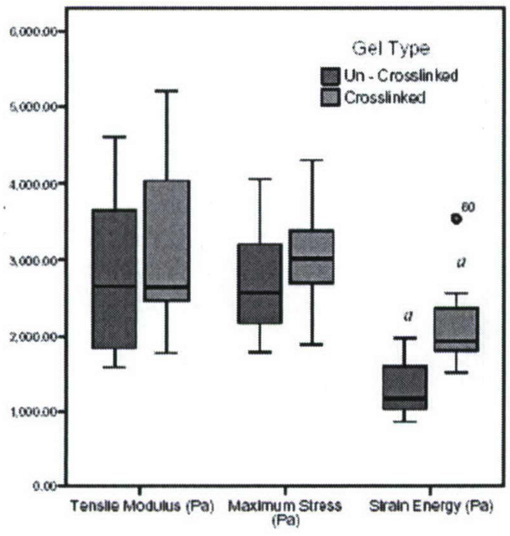


Figure 6: Tensile modulus, ultimate tensile strength, and strain energy density of control and photocrosslinked collagen gels.

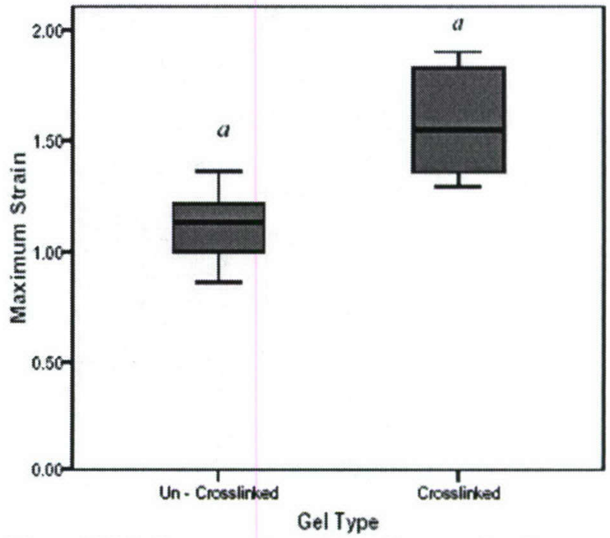


Figure 7: Maximum strain at failure for control and photocrosslinked collagen gels.

Summary of Accomplishments

In an effort to develop tissue engineered interfaces to enhance prosthetic integration, the following significant milestones have been achieved:

- Development of alginate scaffold formulation that delivers bioactive insulin-like growth factor-I (IGF-I) over 4 weeks
- Grafting of the peptide sequence KPLHALL to alginate, which enhanced binding IGF-I to the polymer by 40 fold
- Photocrosslinking of collagen scaffolds using riboflavin as an initiator
- Development of an in vitro model system for evaluating mechanical integration of bone to porous metal prosthetic materials
- Demonstration of enhanced tensile and compressive properties of photocrosslinked collagen gels